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EDITORIAL

A MEDICAL MILESTONE

ALREADY, it appears that the Salk poliomyelitis vaccine will rank among the great discoveries in the field of medicine. While the careful study of its value carried out last summer and recently reported showed it to be less than one hundred per cent effective, it seems quite likely that, with more experience in its production and use, it may well become as valuable as smallpox vaccine as a prophylactic measure.

It would be difficult to assess the sum total of human agony and suffering which polio has brought to civilized man—suffering not only by those afflicted with it, but also suffering in the form of mental anguish on the part of parents, either in fearing for their child or seeing it brought down with a disease at times leaving the child a permanent cripple. The prolonged sorrow of seeing one's child crippled beyond correction and struggling to compensate for the infirmity is something that cannot be appreciated except by those parents so unfortunate as to have endured it. In some measure, sudden death on the part of a loved one is more merciful than this, both to child and parent alike. Now, all of this may be past since the vaccine seems to prevent serious paralytic complications, as well as death so often caused by the bulbar type of the disease.

This apparent conquest of poliomyelitis is a good example of what can be done by teamwork on the part of scientists when properly backed, financially and otherwise, by an enlightened and aroused public. Is it not possible that all of the great problems of prophylaxis and therapy which still remain also can be conquered by a similar approach? Poliomyelitis is a disease which has struck fear into the hearts of all for many years. It was dramatized by the late President Roosevelt in his heroic efforts to overcome its effects and lead a normal life in spite of its ravages. It was indeed he who mobilized public opinion and support which led to the National Foundation for Infantile Paralysis. The work of this Foundation over the years has been a major factor not only in relieving

the victims of the disease, but in organizing the effort leading to its conquest. It has been the success of this Foundation all along the line which has caused the formation of similar groups directed against other perplexing and dangerous diseases.

The ingenuity of man and his indomitable will when properly inspired and mobilized cannot fail. This in itself seems to be one of the many indications that, in each human, there is something of the Divine. Challenges and difficulties which would prove insurmountable to lesser creatures are within the realm of possibility, if only humans would recognize their inner power and use it wisely.

Man, undoubtedly, has not begun to reach that stage of development which is his destiny. It is in such instances as the present conquest of poliomyelitis that the true greatness of man and his potentialities come into full view. Just as early man could not possibly conceive the nature of his descendants in these far distant times, so we cannot appreciate all that man and civilization shall be when we have reached our final destiny. In those days, we have no doubt but that disease no longer will be a matter of great concern. There will, of course, be other hazards and, possibly, of even greater significance—but they will not be caused by micro-organisms.

In closing, we wish to pay high tribute to all those scientists making Salk's final achievement possible, to Dr. Salk himself, and to the pharmaceutical industry which has translated his laboratory experiments into a product that will protect many millions of our children and young adults.

L. F. Tice

THE NATURE OF POLIOMYELITIS VACCINE
A Review

By Morton E. Goldberg *

Introduction

POLIOMYELITIS is an acute infectious disease of virus etiology characterized by a febrile course with evidences of central nervous system involvement; particularly of the spinal cord. It is often accompanied by the development of flaccid paralysis of irregular distribution and extent in a variable proportion of cases. It appears to be the most dreaded of all infectious diseases that attack children in the summer months. Although many cases of polio have arisen in the last few centuries, it wasn't until 1840, that a German physician named Heine first described the disease as an entity, separating it from other forms of paralysis and paralytic diseases. In 1896, Caverly showed that the disease is possible without concommitant paralysis.

As to its epidemiology, it is world wide in its distribution, but much greater than half of all cases reported are from North America, especially the United States. It is believed that some cases of paralysis reported in the Bible may have been due to poliomyelitis. Severe epidemics have occurred sporadically in this country since 1900. The years 1907, 1916, 1925, 1930, 1931, and the late 1940's and well into the 1950's have been extremely disastrous years for widespread outbreaks. It can be shown that in certain times one state may be spared and another state relatively nearby rather seriously affected; at other times the situation may be reversed. Throughout the world, the first large outbreaks were reported in the Scandinavian countries, although today cases in North America greatly exceed those in any other area.

In general the first symptoms seen in the disease are fever, prostration, sore throat and headache; followed by tremors, stiff neck and back, reflex changes, and in most instances true paralysis. The physician should suspect every febrile illness during epidemics. The

* Presented at graduate Seminar, Philadelphia College of Pharmacy and Science.

confirmation of diagnosis is usually based upon positive spinal fluid findings. The prognosis is as follows: 75% make a complete recovery with no residual paralysis, 5% of cases terminate fatally; if paralysis lasts more than two weeks, it will probably be permanent.

Classification and Major Symptoms

1. *Abortive*—fever, headache, nausea, vomiting, tonsilitis, pharyngitis.

2. *Non-Paralytic*—a mild onset followed by a symptomless period, followed by more severe symptoms, high fever, frontal headache, vomiting, stiff neck and back, and a positive spinal fluid.

3. *Paralytic*—

a. *Spinal*—weakness or flaccid paralysis of muscles of thorax, abdomen, back, and upper or lower extremities. Most frequent of the paralytic type.

b. *Bulbar-Cranial Nerve Nuclei Group*—dysphagia, regurgitation of fluids from nose, pooling of secretions in throat, nasal speech, inability to cough or talk (may predispose to pneumonia and other respiratory disorders, dyspnea, and cyanosis).

c. *Bulbar-Respiratory Center Group*—variations in rate and depth of respirations, prolonged respiration time, intervals increase; increased periods of apnea, confusion, delirium, and pulmonary congestion.

d. *Bulbar Circulatory Center Group*—rapid, faint pulse, high blood pressure, anxiety, hyperthermia, coma, state of shock.

e. *Bulbar-Encephalitic Group*—anoxic symptoms, excitability, anxiety, restlessness, twitching, muscular tremors, confusion, lethargy, coma, convulsions in children.

f. *Combined Bulbar-Cervical Cord Group*—cranial nerve palsies, cardio-respiratory center involvement, diaphragm, and intercostal involvement.

It is believed that the virus enters the body through the membranes of the nose, and passes along the nerves of smell to the brain and spinal cord, where its main attack is made. It also enters the body via the intestinal tract, the lymphatics, etc.

It is known, in this climate, that the disease is more prevalent in the summer months. This may be due to the fact, as some investigators have suggested, that flies and other insects may act as carriers for the virus and their abundance in the warmer months provides a greater incidence of occurrence.

Vaccines for active immunization against polio are not new. As far back as 1935 and perhaps earlier, attempts were made to produce immunity. Two particular vaccines: the Kolmer Vaccine, which used an attenuated virus; and the Brodie Vaccine, which used an inactivated virus were suggested as prophylactic agents. The former was too dangerous for use, and the latter proved ineffective. These are mentioned only to point out that active immunity against polio is not new.

Transmission of poliomyelitis in animals was first demonstrated in 1908. Shortly after, it was shown that the etiologic agent was a filterable virus. In 1910 it was known that 1) moneys surviving one attack were resistant to subsequent attacks; 2) the serum of these animals possessed the property of being able to neutralize the virus *in vitro*. The realization of these facts suggested that susceptible persons could be protected against infection by blood from donors who were immune. This type of immunity would be a short one, of the passive type. However, success with other viral infections encouraged steps in the production of active immunization. Basically, active immunization consists of the injection of killed or attenuated bacteria or viruses and the subsequent production of antibodies against the specific antigens. Up until this last year, only one decisive experiment on prophylaxis in man was demonstrated. This was a trial of passive immunization using gamma globulin which was prepared from the blood of immune persons and which contained the antibodies in a concentrated form. Results reported by investigators showed that although protection was afforded, it was of short duration, and the supply too inadequate for the needs of the people. It was shown that protection and/or modification of paralytic involvement was most significant at four weeks after injection and appeared to last well into the sixth week and up to the eighth week after the injection. It is easily understood that although gamma globulin is of value, it is necessary that a relatively inexpensive, long-lasting means of active immunization is the only means of eradicating poliomyelitis. Recent advantages of increased knowledge and techniques

have made it possible to prepare vaccines that give to animals a high degree of protection over a substantial period of time, and to thoroughly control the dosage through assay.

Within the last two or three years, a few laboratories have published reports of active immunization against polio, in small groups of humans. As is well known, one particular vaccine, commonly called the "Salk Vaccine," is now of widespread interest, not only to men in research, but to laymen as well.

In March 1953, news from the University of Pittsburgh offered hope of a promising new vaccine, developed by Dr. Jonas E. Salk. This followed by four years the original work of Dr. John F. Enders of Harvard University, who had found a way to grow polio virus in tissue culture in the laboratory. Enders showed, using human cells, that the polio virus is capable of growth on the following: embryonic skin and muscle, intestines, brain, lungs, kidney, amniotic membrane, postnatal prepuce, uterus, thyroid, thymus, testis, and tonsils. He showed a great similarity when using monkey testicular tissue (fibroblasts) and human testicular fibroblasts in the degenerative changes produced by the virus. Shortly thereafter, it was established that the ideal organ for growth of the viruses was the kidney. Up until the time of Ender's discovery, the virus had only been grown in the brain and spinal cord of infected monkeys and mice. Vaccines prepared from nervous tissue of infected animals might be dangerous for human use. The reason for this will be discussed shortly. The amazing experiments of Dr. Enders opened a possible test-tube source of an almost unlimited supply of polio virus. This work stimulated research laboratories everywhere. As early as 1950, Connaught Laboraties in Toronto had developed Mixture 199, a nutrient capable of maintaining cellular life. Independent investigators at Yale and Johns Hopkins found that polio virus circulates for a short time (from a few hours to a few days) in the blood stream of animals before it enters the nervous system. This was the discovery which suggested that a vaccine which would stimulate the production of antibodies in the blood stream might prevent poliomyelitis. This put the wheels of research into motion which ultimately led to the vaccine developed by Dr. Salk.

Types of Poliomyelitis Viruses

In 1949, a grant of over one million dollars was awarded to four co-operating universities to identify the several types of infective polio

viruses. For many years, scientists had suspected that more than one immunogenic type of polio virus was responsible for manifestation of the disease in man. But how many there were, and how they differed was unknown. Nor would it be seemingly possible to launch a definite attack against the problem until years of basic research had provided the necessary knowledge and techniques to attempt a solution. In 1951, as a culmination of studies on hundreds of individual strains of viruses recovered from patients all over the world, it was established that there are three distinct immunogenic types of polio viruses. Tentatively they have been called:

Type I (Brunhilde or Mahoney)

Type II (Lansing or MEF-1)

Type III (Leon or Saukett)

Originally, the first name in parenthesis was the one commonly designated as the type, but recently the numerical classification seems to be more prevalent; and it is suggested that in the future only the numerical system be used. The original Brunhilde virus was originally isolated from the spinal cord of a chimpanzee of that name which was inoculated in 1939 with stool specimens from seven paralytic polio patients from Baltimore. The Lansing virus had been recovered in 1938 from the brain and spinal cord of a young man who had succumbed to polio in Lansing, Michigan. The original Leon virus was first seen in 1937, when recovered from the brain and spinal cord of an eleven-year-old boy, named Leon, who was a victim of the disease in Los Angeles.

It is important to realize that although all three viruses produce identical clinical symptoms, injection by one will not produce the antibodies effective against either of the other two. This explains that in the production of Poliomyelitis Vaccine, all three types are combined in the production of the finished biological product.

Production of Poliomyelitis Vaccine

As some has put it, "From the monkey's kidney to the arm of a child." However, as we shall see, the story is not so simple. The following steps are taken in the production of the vaccine. Later, we shall discuss them in detail.

1. Monkeys are tested, kidneys are removed and minced.
2. Added to Mixture 199, incubated for 6 days.
3. Medium replaced, new one added containing seed virus, incubated 4 days.
4. Harvested in bottles, then cultured for bacteria.
5. Filtered and pooled.
6. Testing procedures.
7. Inactivated with formaldehyde.
8. Tested for inactivation.
9. Safety measures in inactivation.
10. Neutralization of formaldehyde with sodium bisulfite.
11. Further tissue culture testing.
12. Inside and outside checks.
13. Preservative added, and packaged.

Generally speaking, the process does not appear intricate; merely a logical stepwise procedure which consists of growing the virus on cultures of monkey tissue, harvesting it, inactivating it, and packaging it. But, it is highly unlikely that any one knows the amount of money, the hours of research, and the suffering which has brought us finally to our present place in the conquest of poliomyelitis. Let us now discuss the production of the vaccine in detail.

The production of a virus vaccine is not entirely new, in the order of the development and appearance, they are: smallpox, rabies, influenza, and mumps. However, this is the first vaccine which has been produced on a large scale in tissue culture for human use. As has been said previously, the kidneys of healthy monkeys are employed for the culture. Some immediate questions that may arise are:

1) why tissue culture is employed?

2) why is the monkey used?

3) why is the kidney employed?

Many investigators have pointed out, that tissue culture has the following advantages in obtaining an infective agent over that of using the intact animal:

a. is easier and less time-consuming.

b. is cheaper to obtain in large quantities.

c. is obtained in a purer state with less chance of protein carry-over.

The monkey is used because it is well known that the polio virus has a greater tendency to attack the tissues of a primate, and the monkey is the least expensive and the most practical primate to obtain and handle on a large scale. Finally, as to why the kidney is employed. Since the brain and spinal cord are the natural site of involvement of the disease, and since these elements have a larger mass, why shouldn't they be used over the kidney? These tissues are not employed because it has been found that nerve cells have a poor survival rate in tissue culture, and secondly it is not impossible that certain proteins of the central nervous system may be carried over from tissue culture and may act as antigens in some individuals which may cause a condition entitled demyelination encephalitis. This condition is characterized by an inflammation of the brain which is accompanied by a destruction of the myelin sheath of nerves or nerve tracts. Although the virus has been successfully cultured on the uterus or testis of monkeys the use of only one tissue is practical. This would limit the production of the vaccine from either male or female monkeys, and both could not be used.

The organ which is the ideal one is the kidney, this is because renal epithelial cells have an excellent survival rate in tissue culture and that there is a fairly large mass of tissue to work with in the production of the vaccine.

The methods presently employed by the various manufacturers producing the virus are the same. Monkeys are received, housed in a separate dwelling, placed in cages not to exceed fifty to sixty animals per cage, and carefully tested. They are given a tuberculin test twice, once during export and once upon arrival. Since a positive tuberculin test indicates that the animal once had or now has tuberculosis, no chances are taken to differentiate this. Therefore, any monkey with a positive tuberculin test is discarded. Then the monkeys are observed for a period of over a week for any clinical signs of illness or disease. Only clinically well monkeys are used in the production of the vaccine. This may sound simple, but it is certainly quite expensive. No figures are available as to how many ani-

mals are used in a given lot, but it is well known that monkeys carry many types of disease-producing organisms and that a very large percentage of them are discarded for this reason. Presently, it has been estimated that the kidney tissues from one monkey are sufficient for enough vaccine to innoculate about several hundred children. However, refinements are expected to increase this amount greatly.

Now the actual production of the vaccine begins. The monkeys are anesthetized, dipped into a germicidal solution, dried, and the kidneys are aseptically removed. After the kidneys are removed the animal is carefully autopsied. If any hint that a diseased animal was used, the kidneys are discarded, rather than take a chance. The capsule of the kidney is opened and removed. The kidneys are then minced and added to the culture Mixture 199, developed by Connaught Laboratories. This medium is a mixture of more than 60 ingredients, which when properly combined will not only keep tissue alive, but will actually permit its reproduction. Polio virus naturally reproduces only on living cells. Included in this formula are vitamins, minerals, amino acids, and dextrose. Penicillin in a concentration of 200 units/cc. and streptomycin in a concentration of 200 mcg./cc. are employed for their antibacterial activity. This medium is a red fluid and the color is due to the presence of phenol red, which is used as an indicator for pH value. The minced kidney is incubated and allowed to stay in this medium for a period of six days at 36° C.; during this time, the cells adapt themselves to the presence of the medium and proliferate to an optimum degree. After the first six-day incubation period, the medium is removed and replaced with fresh medium which contains the particular immunogenic strain of virus. The strains are cultured separately and only when completed are they mixed together. The culture is once again incubated, this time for a period of four days. During this time the virus undergoes optimum reproduction. Enders, Melnick, and other investigators have demonstrated that the virus titer obtained in tissue culture is much higher than that produced in the intact animal. This titer level has been demonstrated in all types of polio virus. After this time interval, an extensive filtering procedure is employed. Three filters are used, each of increasing fineness, the last one is a Seitz filter which is capable of removing bacteria. Next, a pooling process is undertaken in which the filtered virus is pooled in a large tank. At this stage various tests are performed on the culture for safety reasons. Firstly,

the virus titer of the culture is determined. If it is too low, it is discarded. The absence of a certain virus, which is found in monkeys and which may harm humans, called B virus, is established. This is provided for by rabbit innoculation. The sample is cultured for bacteria, including *Mycobacterium tuberculosis.* A sample is also injected into guinea pigs for the determination of the above. If all the above tests prove favorable, the important process of inactivation is begun. During this stage, it is desirable to destroy the disease-producing power of the organism, but at the same time ensure its antigenic properties. In the past, it has been shown that biologicals can have their infective properties destroyed by either heat or formaldehyde. Experiments by Salk and his associates have shown that formaldehyde is the more favorable agent. The variables that influence the time required for the destruction of infectivity are:

1) concentration of free formaldehyde.
2) temperature of the reaction.
3) pH of the reaction mixture.
4) the concentration of the virus originally present.

While most of the above variables can be controlled, if any one of them varies from time to time, the actual time for inactivation will vary. The conditions selected from the above considerations are:

1) 1:4,000 formalin solution (represents 0.009% HCHO, assuming formalin solution contains 37% HCHO).
2) a temperature of 36-37° C.
3) a pH of 7.

When these conditions are kept constant, then the time required for inactivation, varies directly with the original titer of the virus. Also as Salk has pointed out, if the protein or amino acid content of the culture varies, either of which is known to bind formaldehyde, then the inactivation time will be varied. Another consideration which concerns the safety and effectiveness should be mentioned. It is related to the possibility that tissue fragments, or isolated cells, or even cell debris, might be present in the suspension, and that these might contain entrapped or adsorbed virus, that might not be readily accessible to the formaldehyde as are the virus particles that are free

in suspension. This is avoided by the exhaustive filtering process mentioned previously, which prevents this from occurring.

The inactivation process is interrupted periodically to determine the amount of residual virus. For additional safety, the inactivation period is continued for a total of three times the period necessary for complete disappearance of virus infectivity; an effective means by which the virus potency is tested, is with the use of trypsin, added to a cell suspension and then stirred in a Waring Blendor. The trypsin is capable of destroying the intercellular cement substance without injuring the individual cells. Once this has occurred, the virus is titrated with type specific antiserum to determine its infective power. Some finer points of the inactivation process should be mentioned. Usually after filtration as previously mentioned, the free carbon dioxide in solution in the culture fluid, as a result of tissue respiration, is removed by transfer from one container to another, under a partial vacuum in a closed system. This is done, in order to adjust the pH to a finer degree so that it will remain constant during inactivation. The pH of the carbon dioxide free culture is approximately 8.3-8.4 and is adjusted to a pH of seven by the addition of either 0.1 N hydrochloric or acetic acid.

A pH of 7 is employed since at this level slight changes in pH are not too detrimental to the finished vaccine. A slight increase in pH at a level of 8.3 is.

It is interesting as to the reason why such a small concentration of formaldehyde is used, since a larger concentration will reduce infectivity in a much shorter time. The reason for this is that the inactivation at the lower concentration results in better retention of antigenic activity. Although stability of the vaccine has not yet been definitely established, it is believed that it should be one of the more stable products among biologicals.

The method used to determine when the inactivation period should be terminated is as follows: A graph is drawn, plotting per cent infectivity against time in hours after the formaldehyde is added. The time intervals begin immediately upon the addition of the formaldehyde. After a titer level is reached which in a 1:10 dilution fails to produce clinical manifestations, a line is connected to that point, and through the initial determination. (This gives the longest theoretical inactivation time.) The inactivation period is extended for three times the theoretical inactivation time of zero infectivity.

After the inactivation period is terminated, the formaldehyde is neutralized with sodium bisulfite. Some controversy has arisen over this point since it has been shown with certain other antigens that the presence of a low concentration of formaldehyde tends to maintain stability rather than diminish it. However, as Salk has pointed out, until this can be studied in relationship to the poliomyelitis virus, it is deemed desirable to continue to neutralize the excess formaldehyde.

After this neutralization process, the material is tested to rule out the presence of residual infectious virus in any quantity whatsoever. The tissue culture method is used; however, very much larger quantities of virus culture are used, to ensure detection of the virus if present. If no residual virus is found, the three individual strains are pooled to form the mixed vaccine. The three strains are cultured separately because of differences in growth rate and slight modification in testing procedures. Numerous tests are once again repeated on the vaccine, these tests include beside the tissue culture test, an additional test for residual active virus by innoculation into the brain of monkeys, and mouse innoculation is done for the presence of a particular virus causing lymphocytic choriomeningitis. Also the vaccine is cultured for the presence of molds and bacteria. Finally the antigenicity is determined by injection into monkeys and mice, and the subsequent test of antibody titer of the animals serum. Next, an outside check is done. Samples of the vaccine are sent to the National Institutes of Health for a repetition of the tests thus far mentioned.

The antigenicity tests which are performed on the finished vaccine are done in two ways. In the first test six monkeys receive three doses of the vaccine at weekly intervals, at the fourth week the serum is collected, and tested in serial dilution for its ability to neutralize standard amounts of the three strains of viruses in tissue culture. In the other antigenicity test, the vaccine is injected into mice intraperitoneally, and at a predetermined time, the mouse serum is pooled, mixed with a graded amount of active virus, incubated for a period of time, and then innoculated into untreated mice. Since type II virus is generally attracted to the brain of animals, it is injected intracerebrally into some mice, and intraspinally into some others because of the tendency for types I and III to attack the spinal cord. However antigenicity in animals is unimportant, if it does not apply to humans also. That the vaccine is non-infective seems

reasonably sure; whether it has prolonged protecting ability is another thing entirely.

In summary then the following safety measures are taken in the manufacture of the vaccine:

1. tuberculin tests.
2. careful autopsy.
3. sterility tests for medium 199.
4. sterility tests for virus by bacterial culture.
5. tests of presence of *M. tuberculosis.*
6. tests of presence of B virus.
7. samples taken during inactivation to determine infectivity.
8. inactivation for 3 $\times$ necessary time.
9. tests on individual strains for presence of certain viruses.
10. vaccine injected intracerebrally in 12 Rhesus monkeys and intramuscularly in 6 Cynomolgus monkeys as additional test.
11. tests for presence of virus of lymphocytic choriomeningitis.
12. antigenicity tests in monkeys and mice.
13. outside check at National Institutes of Health, Laboratory of Microbiological Control.

The trial of the Salk vaccine began last April under the auspices of the National Foundation for Infantile Paralysis. The results have been evaluated by Dr. Thomas Francis, Jr. of the University of Michigan, School of Public Health. The report of this study given on April 12, 1955 showed the vaccine to offer considerable protection, particularly against the more severe forms of poliomyelitis.

The Foundation announced on April 12 the result of the tests on the vaccine and it was an unqualified success.

The vaccine program cost the National Foundation for Infantile Paralysis $26,500,000 for the trials along with the gamma globulin project, a preventive one.

National Foundation officials were so hopeful of the Salk Vaccine, they allocated in advance $9,000,000 of the funds collected last year. The money was considered a calculated risk to set up a stockpile of the vaccine in the event it was found effective and to have it ready to provide three injections immediately to every first and second grade child in the United States. Others may be immunized by vac-

cine obtained through regular pharmaceutical channels. The product has been released by the National Institutes of Health for commercial distribution and six of the major pharmaceutical houses are engaged in its manufacture.

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MECHANISM OF THE ANTI-INFLAMMATORY ACTION OF TRYPSIN

By Gustav J. Martin, R. Brendel, and J. M. Beiler *

EVIDENCE has been reported (1) indicating that the inhibitory action of trypsin in egg-white edema is not primarily concerned with the catalysis of the conversion of profibrinolysin to fibrinolysin. This finding raised the question as to whether some other indirect mechanism is concerned, or whether the enzyme acts directly to facilitate drainage at the site of edema. The results reported below suggest that trypsin has a direct mode of action, and that its anti-inflammatory effect is the result of a combination of several different physiological actions.

Experimental

Testing of the extent of edema inhibition was carried out by the technique previously reported (1). Except where otherwise specified substances tested were injected in two doses, ½ hour before and simultaneously with the injection of egg-white. Tissue homogenates were prepared by treating the appropriate rat tissue with 5 volumes of distilled water in the Waring blendor, and incubated for 4 hours at 37° with trypsin at a concentration of 0.3 mg./ml. Oxalated whole blood and plasma were used, concentration of trypsin for incubation being the same as that for the tissue homogenates.

Results and Discussion

All results reported are averages for groups of 5 animals, each experimental group being run with its own control. The figures in Table I show that in no case was inhibition of edema obtained with tissue homogenates pre-incubated with trypsin. Blood, plasma and serum caused some inhibition, suggesting the presence of an anti-inflammatory factor, but in no case was this effect heightened by trypsin pre-treatment; indeed, it appeared to be counteracted. The negative results obtained with these trypsin-treated tissues do not of course exclude the possibility that in the body trypsin acts on some tissue to produce proteolytic products which in turn modify the ex-

* National Drug Co. Laboratories, Philadelphia, Pa.

perimental edema. However, the figures presented in Table II show that trypsin when injected with the egg-white directly into the site of edema is effective at an appreciably lower dosage than when it is administered at other points.

TABLE I

EFFECT ON EGG-WHITE EDEMA OF TISSUES PRE-INCUBATED WITH TRYPSIN

Tissue	*Weight Difference Saline Leg-Egg-White Leg (gm)*	*Inhibition (%)*
Liver	1.92± .07	0
Control	2.15± .12	
Kidney	2.05± .07	0
Control	2.15± .12	
Spleen	2.12± .12	0
Control	2.15± .12	
Muscle	1.92± .07	0
Control	2.15± .12	
Blood	1.42± .07	30
Control	2.12± .11	
Blood & Trypsin	1.60± .05	25
Control	2.12± .11	
Plasma	0.76± .04	45
Control	1.42± .14	
Plasma & Trypsin	1.35± .12	0
Control	1.42± .14	
Serum	1.50± .15	20
Control	1.95± .12	
Serum & Trypsin	1.70± .15	0
Control	1.95± .12	

Dosage 0.6 ml. in 2 doses ½ hour apart throughout.

The most logical interpretation of this finding is that trypsin acts directly on the edema without the involvement of any intermediate reaction, and that administration at the site of edema simply provides a higher local concentration at the point where the enzyme acts. It is worthy of mention that the presence of a specific trypsin inhibitor in the egg-white may affect the activity of the enzyme when it is administered in this fashion, so that the figures may be taken as representing a minimal effect.

TABLE II

EFFECT OF PHYSIOLOGICALLY ACTIVE AGENTS ON EGG-WHITE EDEMA

Agent	*Dose*	*Wt. Difference Saline Leg-Egg-White Leg (gm)*	*Inhibition (%)*
Trypsin	1 mg/K (1 dose s.c.)	1.77± .12	0
Control		1.80± .05	
Trypsin	1 mg/K (1 dose with egg-white)	1.35± .15	30
Control		1.90± .05	
Hyaluronidase	400 TRU (with egg-white)	1.55± .17	25
Control		2.05± .12	
Hyaluronic Acid	10 mg/K (with egg-white)	1.65± .12	0
Control		1.80± .05	
Hyaluronic Acid (Partially depolymerized)	10 mg/K (with egg-white)	1.35± .20	25
Control		1.80± .05	
Mecholyl	2.0 mg/K (1 dose s.c.)	.75± .25	60
Control		1.85± .05	
Heparin	(3,000 Units/K (1 dose IP))	1.80± .10	0
Control		1.94± .15	
Trypsin	1 mg/K (2 doses s.c.)	1.40± .18	0
Control		1.48± .07	
Chymotrypsin	1 mg/K (2 doses s.c.)	1.85± .20	0
Control		1.90± .09	
Trypsin & Chymotrypsin	1 mg/K each (2 doses s.c.)	1.30± .25	35
Control		2.02± .15	

Table II gives the results obtained with several compounds which were tested because of similarities in their physiological action to that of trypsin. Hyaluronidase has been reported (2) to inhibit the inflammatory reaction by causing an increase in vascular permeability and thus facilitating drainage. Partially depolymerized hyaluronic acid has also been reported to increase permeability (3). Both of these substances were effective in inhibiting egg-white edema. Both will of course facilitate intradermal dye spreading. Trypsin, as well as other proteolytic enzymes which inhibit this edema (1) also has this effect; proteolytic enzymes inactive against egg-white edema have no effect on interdermal dye spreading (4).

Trypsin causes vasodilatation (5). Mecholyl, a potent vasodilator, had a pronounced effect on the experimental edema. On the

other hand heparin, administered ½ hour before the production of edema in sufficient concentration to render the blood incoagulable during the course of the experiment, was without inhibitory effect. A combination of trypsin and chymotrypsin at concentrations of each enzyme which individually had no effect was markedly inhibitory. These two enzymes are known to reinforce each other's proteolytic action (6), and the effectiveness of the combination in inhibiting the development of egg-white edema may be related to the observation that trypsin produces a decrease in viscosity of the body fluids (7).

It would thus appear that the anti-inflammatory action of trypsin is a result of a direct facilitation of drainage from the inflamed area. The abilities of trypsin to increase permeability, cause vasodilatation and reduce viscosity of edema fluid will all have the effect of facilitating drainage. Agents which have these properties individually will inhibit the experimental edema.

Summary

Evidence is presented suggesting that the anti-inflammatory effect of trypsin is the result of direct action on the inflammatory process. Possible mechanisms for the action are discussed.

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WIDENING HORIZONS IN ENDOCRINE THERAPY *

By W. W. Swingle **

SO many new and important advances have been made in the field of medicinal chemistry in recent years that those engaged in the profession of pharmacy, along with the physician and biologist, have found it necessary to acquire a new scientific vocabulary. Today, not only the research pharmacist but also the pharmacist behind the counter at the corner drug store must have some knowledge of a host of drugs and scientific terms undreamed of a generation ago. The constantly widening horizon of therapeutics has rendered it imperative that the student of pharmacy keep broadening the base of his specialty in order to cover the recent discoveries.

The subject of steroid hormones will serve to illustrate the startling rapidity with which a field of interest to the pharmaceutical profession has developed and immensely widened the area of clinical control and rational treatment of many and varied human disabilities.

Just twenty-five years ago Doisy and his co-workers announced the isolation in pure form of the first known steroid hormone. This was estrone which they obtained from the urine of pregnant women. Between 1929 and 1936 knowledge of the sex hormones was greatly extended and within a few years the three general types of reproductive hormones, viz., the estrogens, androgens and progesterone had been crystallized, their structure determined and their synthesis accomplished.

The clinical application of the newer knowledge and understanding of the sex hormones to the disabilities affecting the reproductive process constitutes a brilliant chapter in the history of therapeutics and is one that is well known to all of us. The endocrine therapy of sexual infantilism, disorders of menstruation, threatened abortion and menopausal symptoms has by no means solved all of the problems involved in the treatment of these disorders but the availability of the pure hormones has surely eased the sufferings of great numbers of mankind.

Study of the chemistry of the steroid hormones of the adrenal cortex began in 1935 and proceeded so rapidly that within five years

* Based on a lecture given at the Columbia Bicentennial Conference, October 15, 1954.

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28 crystalline steroids had been isolated and characterized. The 29th member of this surprising array of cortical principles was only crystallized in 1953 and its identity established in the spring of 1954. However, of the 29 compounds but 7 are definitely known to be active in maintaining life of adrenalectomized animals. Structurally they are much alike with the exception of aldosterone and differ from one another by the number and nature of the oxygen functions borne by C_{11} and the side chain at C_{17} and at C_{21}. Aldosterone, the most recently isolated member of this group of physiologically active steroids differs from the others in having an aldehyde function substituted at C_{18} of the angular methyl group. According to Reichstein, when the compound is in solution there is probably a balance between its two forms with one predominating.

It may be of interest to recall the difficulties under which the early investigators labored in isolating these compounds, by citing some figures on the yields obtained by Kendall, Reichstein, Wintersteiner and Pfiffner and their respective research teams in their fractionation studies of beef adrenals. Starting with 1000 pounds of glands the following quantities of adrenal steroids were recovered.

Dehydrocorticosterone	330 mg.
Cortisone	340 mg.
Corticosterone	180 mg.
Desoxycorticosterone	12 mg.
Aldosterone	22 mg.
Hydrocortisone	Less than 100 mg.
11-Desoxycortisone	Less than 100 mg.

The low yields of crystalline material made it imperative that these hormones be synthesized from cheap starting material. Indeed this became a most pressing problem when in 1949, Hench, Kendall, Slocumb and Polley announced the results of their studies on the value of cortisone in rheumatoid arthritis thereby demonstrating the enormous clinical possibilities of adrenal C_{11}-oxygenated steroids. The medical profession, the experimentalist and indeed the human race owes a debt of gratitude to the pharmaceutical industry for their efforts in making these compounds readily available for clinical use. The synthesis of the first small sample of cortisone from desoxycholic acid was accomplished by Sarett in December 1944 while working in

the laboratories of Merck and Company. The synthesis was reported in 1946, and Merck released the first few grams of cortisone for clinical testing in 1948. The following year a small quantity of hydrocortisone isolated from adrenal cortical extract was supplied by The Upjohn Company to the Mayo Clinic for testing. More recently this steroid was synthesized by Tishler and his associates in the Merck laboratories and today both cortisone and hydrocortisone are in common use.

Desoxycholic acid obtained from ox bile was the first and still remains the initial starting material for commercial production of cortisone. However, within the past few years new synthetic methods have become available so that it is possible to produce adrenal cortical hormones from more common and hence cheaper steroids such as cholesterol and ergosterol; the Mexican Yam, Dioscorea, has also yielded source material.

Even with the newer synthetic processes and abundance of starting material, the synthesis of the more important cortical hormones such as cortisone and hydrocortisone involves the difficult chemical task of introducing an oxygen function into the steroid nucleus at C_{11}. This synthetically arduous feat can be accomplished by the use of microorganisms; certain actinomyces and mucorales organisms have the capacity, during the process of fermentation, of introducing an hydroxyl group at C_{11} in a variety of steroids. By so doing, they can transform a steroid whose principal function is control of salt and water distribution in the body into a steroid concerned primarily with certain phases of protein and carbohydrate metabolism.

The important work of Peterson and Murray of The Upjohn Company illustrates the simplicity with which microbiological hydroxylation of steroids can be achieved. They took progesterone and added it to a special nutrient broth which had been seeded with a type of common mold. This culture medium was permitted to ferment for 24-48 hours, the steroid was then removed and found to have been changed to a new C_{11}-oxygenated intermediate which could then be converted to either cortisone or hydrocortisone by well-known chemical reactions. Several other similar steroid transformations have been reported following the use of molds. For example, 11-desoxycortisone (Compound S) which is a comparatively weak mineralocorticoid can be transformed by microbiological hydroxylation into hydrocortisone, the most potent of the glucocorticoids.

The dream of the steroid chemist has finally become reality since the total synthesis of the most important adrenal steroids from relatively simple organic compounds has been accomplished. In 1952, Woodward and his co-workers announced the formation by total synthesis, of an intermediate compound of steroid nature from which it is possible to proceed using established reactions, to cholesterol, testosterone, cortisone and other known steroids.

Sarett and his colleagues in 1952 working at Merck and Company, also announced the total synthesis of cortisone from comparatively simple organic molecules.

Despite the fact that the skill of the organic chemist has enabled him to synthesize the adrenal cortical hormones from small molecules, knowledge concerning the mechanisms by which the cortex elaborates steroid hormones is far from complete. Brilliant contributions to the problem of steroidogenesis within the cortex have been made by Pincus, Hechter and Zaffaroni. These investigators devised an ingenious method for production of adrenal steroids *in vitro* by perfusion of the adrenals of beef and other animals. They took the glands of freshly killed animals and placed them in a perfusion apparatus. The tissue was kept alive by pumping citrated whole blood of beef through the gland by a device which simulated the rhythm and pulsation of the actual arterial pressure. Oxygen was added for aeration plus penicillin and streptomycin to keep the material sterile. The whole apparatus was maintained at body temperature. The amount of blood perfused varied from 200 to 2000 cc. and could be re-circulated as many times as desired. The isolation of the neutral steroid fraction from the perfusate by adsorption on charcoal, subsequent elution with organic solvent and application of paper chromatographic techniques made it possible to identify the individual corticosteroids. By means of this technique, Pincus, Hechter and Zaffaroni were able to analyse the effluent from the perfused glands for secretory products and to study the factors which influence the nature and quantity of these products. They could add known steroids to the perfusate and after circulating the blood through the gland and studying the effluent, determine the chemical transformations which the gland was able to effect. In numerous experiments it was found that steroids such as desoxycorticosterone which lacks an oxygen at C_{11}, could be transformed into corticosterone which has an oxygen at C_{11}, or Compound S (11-desoxycortisone) which also has no

oxygen at this position could be converted to hydrocortisone. It is now an established fact that the isolated adrenal gland has the capacity to hydroxylate preformed steroids not only at the C_{11} position but also at C_{17} and C_{21}. Steroid biogenesis in the perfused gland was greatly speeded up by the addition of corticotropin to the perfusate.

Further research has shown that the intact adrenal gland is not necessary for the hydroxylation reactions for it has been demonstrated that incubation of desoxycorticosterone with either slices or homogenates of beef adrenals transforms this compound into hydrocortisone. The enzyme systems involved in these transformations remain unknown as does also the answer to the problem whether or not tissues other than the adrenal possess the ability to effect these hydroxylations. However, it is a safe assumption that the pathway by which the *in vivo* biogenesis of adrenal steroid hormones is accomplished will probably soon be established.

Considering the large number of steroids that have been isolated from the cortex, it becomes important to determine which ones are actually elaborated by the gland and secreted into the blood and which are artifacts resulting from the chemical procedures employed in the isolation process.

Fractionation studies of the venous blood coming from the gland of living animals shows that the chief steroids released are corticosterone and hydrocortisone or else a mixture of both. These two steroids have been demonstrated to occur in the greatest amount in the venous blood from the gland of living animals in all mammalian species so far investigated. The adrenals of monkeys and sheep secrete principally hydrocortisone, those of rats and rabbits produce mainly corticosterone, whereas cats, dogs, ferrets and cows elaborate mixtures of both types of steroid. The adrenals of man appear to secrete chiefly hydrocortisone and probably small amounts of corticosterone. Cortisone has rarely been found to be present and then only in minute amounts. Sweat and his coworkers, studying the adrenal venous effluent of man during surgical operations found the gland secreted 80 μg. of corticosterone and 290 μg. of hydrocortisone per 100 cc. of blood. The adrenal vein blood of a patient suffering from hyperadrenal corticalism or Cushing's syndrome contained 236 μg. of corticosterone and 850 μg. of hydrocortisone per 100 cc.

Quite recently a number of investigators in England and in this country have isolated a powerful sodium-retaining substance in adrenal vein blood of various mammalian species which is probably the newly isolated adrenal steroid, aldosterone. Thus it seems that the chief and perhaps the only steroids normally released by the adrenal cortex are hydrocortisone, corticosterone and presumably aldosterone.

At this point, I should like to restrict the remainder of this discussion to 1) those adrenal steroids which have proven of greatest value therapeutically such as desoxycorticosterone, cortisone and hydrocortisone; 2) to outline briefly quite recent developments such as the isolation of aldosterone and discovery of the surprising physiological activity of the 9-halo derivatives of hydrocortisone.

Desoxycorticosterone was synthesized by Steiger and Reichstein in 1937 before it was known to exist in the glands. The following year Reichstein succeeded in isolating it from beef adrenals. This synthetic steroid has been of great value to the experimentalist working with adrenalectomized animals and to the clinician for use as substitution therapy in controlling Addison's disease. Until recently it ranked first in potency among adrenal hormones for its effect upon mineral metabolism and maintenance of normal health and vigor of adrenalectomized animals and Addisonian patients. It exerts profound renal effects upon sodium retention, potassium excretion and internal distribution of water and certain electrolytes. In fact the potency of this hormone is so great that serious overdosage effects may occur in man and animal. In improperly controlled doses especially when the salt intake is not restricted, the compound may cause hypertension, oedema, increased blood volume, substitution of sodium for potassium in skeletal and heart muscle cells and other disabilities. In the early days of desoxycorticosterone therapy, before the limitations of the steroid were realized, the Addisonian patient was sometimes in as much danger from the treatment as from the disease. However, due to the labors of clinicians, physiologists and research pharmacists the steroid has now taken its rightful place along with other hormones in the armamentarium of the physician. Incidentally it should be noted that much of our present knowledge of the clinical use of desoxycorticosterone in the treatment of Addison's disease and the relation of this steroid to electrolyte metabolism we owe to the researches of an outstanding group of investigators working in the College of Physicians and Surgeons of Columbia University. I refer

to the classical studies of Loeb, Atchley, Benedict, Ferrebe, Ragan and Perera.

Although desoxycorticosterone was a boon to patient and physician, it was soon realized that it does not completely substitute for the adrenal cortical secretions. It lacks an important function, viz., ability to maintain adequate carbohydrate reserves in fasted adrenalectomized animals and Addisonian patients. It is also unable to sustain such animals or patients when they are subject to stress. Since the compound is practically insoluble in water and must be used in oily solutions it is limited in its effectiveness during periods of adrenal crisis. Such individuals are desperately ill and hence radical remedial measures are necessary involving intravenous salt, glucose, fluid and cortical extract in large quantities. To meet this shortcoming of the steroid, the pharmaceutical staff of the Ciba Company in Basle undertook to solubilize the compound in an aqueous medium for use intravenously. Their efforts succeeded and now it is possible to obtain the water soluble glucoside of desoxycorticosterone. The material is non-toxic in so far as my coworkers and I have been able to determine in large scale experiments on normal and adrenalectomized dogs. It is highly effective by vein, in our experience, the most effective way to revive animals lacking adrenals from severe insufficiency. It exhibits only mild overdosage effects even when administered in enormous doses since there is no cumulative effect due to slow absorption of an oil solution. European physicians have reported using the material successfully in Addison's disease but to my knowledge it has not been widely tested on patients in this country.

The demonstration by Hench, Kendall, Slocumb and Polley of the efficacy of intramuscular injections of fine crystalline suspensions of cortisone in rheumatoid arthritis aroused interest in testing suspensions of other adrenal steroids. The Ciba Pharmaceutical Company prepared the trimethylacetate and phenylacetate of desoxycorticosterone and it was shown by Meier that the trimethylacetate preparation when given in oil as a single intramuscular injection of 10 mg. would maintain the adrenalectomized dog in good health for 35-40 days. Thorn made a clinical test and reported that 60-120 mg., when injected intramuscularly into an Addisonian, controlled sodium metabolism for two months. These observations stimulated workers in my laboratory to study both the trimethylacetate and phenylacetate of desoxycorticosterone in adrenalectomized dogs.

Briefly stated our experiments showed that a single injection of a crystalline suspension of 10 mg. of the trimethylacetate was sufficient to keep the animals active, healthy and entirely free from symptoms of adrenal insufficiency for periods ranging from 50-60 days. Similar injections of the phenylacetate maintained the dogs symptom-free for 40-46 days. The animals remained lively, ate full rations and maintained their weight until a few days before termination of the test. Various other crystalline suspensions of desoxycorticosterone and other steroids were tested but none gave results on life maintenance at all comparable to the trimethyl and phenyl acetates. Gaunt, Leathen and associates have performed similar experiments using adrenalectomized rats. To my knowledge none of these desoxycorticosterone preparations are effective in diseases other than those due to failure of the adrenal glands such as occurs in Addison's disease.

A new and significant addition to knowledge of the chemistry and physiology of the cortex during the past year, has been the isolation and determination of the structure of aldosterone. It has long been recognized that after removal of all of the known crystalline compounds from cortical extract concentrates, there still remained in the mother liquor an active, non-crystalline residue designated simply as the amorphous fraction. Interest in determining the nature of this material was revived and greatly stimulated by the experiments of Tait, Simpson and Grundy published in a series of papers between 1952-1953. These investigators devised a simple and rapid method for determining in a quantitative manner, the sodium retaining activity of adrenal steroids on adrenalectomized rats. Using their method of assay they demonstrated the presence of a powerful sodium-retaining substance in adrenal vein blood; similar material was also found in extracts of beef glands which upon assay proved to be twenty-five times more potent than desoxycorticosterone acetate which up until this time was regarded as the most powerful sodium-retaining steroid. Recently other workers in this country have obtained evidence for the presence of a similar substance in the urine of children with nephrosis and in blood from the adrenal vein of dogs. These studies clearly indicated the existence of an unknown hormone presumably steroidal in nature which possessed surprising mineralocorticoid activity.

Simpson and Tait, in collaboration with the Swiss chemists Reichstein and his group and Wettstein and his coworkers, soon isolated

a pure crystalline compound from adrenal extracts which upon assay exerted an effect on sodium retention far greater than that displayed by desoxycorticosterone acetate. The compound was provisionally designated as electrocortine and in the first reports was said to be 30-100 times more active than DCA depending upon the test and animal employed. Two groups of investigators in this country announced the results of their studies on the sodium-retaining factor only a few weeks following the publication of the paper by the British and Swiss workers. Mattox, Mason and their associates at the Mayo Clinic, obtained crystals which possessed mineralocorticoid activity equal to twenty-five times that shown by DCA. Knauff, Nielson and Hains, working at The Upjohn Company obtained non-crystalline but extremely potent sodium-retaining material.

The chemical constitution of the new steroid was established by the Simpson, Tait, Reichstein group, and aldosterone was suggested as a definitive name for the compound. There is general agreement that it is far more active than DCA in tests employing sodium retention as the criterion of potency. The Princeton group using crystalline material supplied by Reichstein, found the average maintenance dose of aldosterone to vary from 6.2-12.5 μg./dog/day when given in 10 per cent aqueous alcohol to adrenalectomized dogs. It was 12-25 times more active than desoxycorticosterone solubilized in a similar manner, and 35-50 times more potent than DCA when the latter was administered in oil. By comparison, the minimum maintenance dose of solubilized cortisone and hydrocortisone for dogs lacking adrenals was 5000 μg./dog/day. This is 500 times the maintenance dose requirement for aldosterone.

The newly isolated steroid differs physiologically in certain respects from DCA: 1) It is far more potent in retaining sodium; 2) It is less active than DCA in stimulating potassium excretion; 3) It has been reported that aldosterone is 30 times more active than DCA in increasing the liver glycogen of mice. Gaunt found it as potent as cortisone when used on rats in the cold-stress test but it appears to be weaker than cortisone in the eosinophile depletion test. The reviewer is familiar with but one detailed published clinical trial of this steroid. It proved to be highly effective in rendering two Addisonian patients symptom-free and the required dose varied between 150-200 μg. (2.5-3.3 μg./Kg./day). The symptoms disappeared a

few hours after treatment began and no signs of edema or other evidence of toxicity appeared.

In a paper as yet unpublished, Simpson and Tait have made some interesting observations on aldosterone in man. They found that the level of circulating steroid per 100 cc. of whole blood is approximately 0.08 μg. and that 20 μg. administered to a normal man will markedly depress the Na/K rates in salivary secretion in 2.5 hours. Finally they showed that aldosterone is as effective when given orally as when given by vein and that 80-100 μg./day, orally controls sodium and potassium levels of the blood in the Addison's diseased patient. Patients with rheumatoid arthritis have been given 200, 400 and 800 μg./day for 6 days. Apparently there was no amelioration of symptoms during this interval. However, there was a moderate increase in weight indicating water retention.

Today hundreds of thousands of dollars are being spent by the pharmaceutical industry, *e.g.*, Ciba, Merck and The Upjohn Company in an effort to synthesize aldosterone and make it available to the clinician as quickly as possible.

The demonstration by Hench, Kendall, Slocumb and Polley that cortisone exerts dramatic therapeutic effects on rheumatoid arthritis patients, initiated a new era in adrenal hormone therapy. Prior to their announcement, cortical steroids had been shown to be of great value in the treatment of Addison's disease but their efficacy in other disabilities remained unproven or of questionable value. Today, few fields of medicine or physiology are more active than those concerned with studies of the functional significance of cortisone in health and its therapeutic value in disease. In spite of the enormous volume of clinical and laboratory data collected, the essential function of cortisone and the other C_{11}-oxygenated adrenal steroids remains unknown. Their action is so widespread, so ubiquitous and covers such an astonishing range of biological functions that our present day knowledge concerning them is still too fragmentary to permit one to formulate an all-embracing theory of their primary function and locus of action.

The physiological activity of those adrenal steroids such as desoxycorticosterone which lack an oxygen at C_{11} differs from steroids such as cortisone and hydrocortisone which have an oxygen at this position. These two compounds exert little control of electrolyte and water distribution but instead are active in functional areas such as

regulation of certain aspects of organic metabolism where the desoxy compounds are relatively inert. Thus cortisone plays an important role in the intermediary metabolism of carbohydrate, stimulates gluconeogenesis, inhibits undue utilization of glucose, maintains the work capacity of skeletal muscle, exerts strong anti-insulin effects, prevents the occurrence of hypoglycemia in fasted Addisonian patients and influences the metabolism of protein and fat. This steroid also increases resistance to stress, maintains the functional reactivity of the peripheral vasculature and suppresses inflammatory reactions of connective tissue. Overlapping of function does occur between the desoxy type of steroid and the C_{11}-oxygenated type. This is illustrated by aldosterone and especially by the 9α-halo derivatives of cortisone and hydrocortisone which exhibit the functional activity characteristic of both steroid types.

The surprising adrenocortical activity of these 9α-halo derivatives of cortisone and hydrocortisone is another new and highly important development in the field of adrenal steroids, made all the more interesting because these compounds are man-made modifications of the natural steroids and apparently superior in potency to the secretion of the cortex itself. This series in which the 9α-hydrogen atom is replaced by halogen, was prepared and tested by Fried and Sabo in the laboratories of E. R. Squibb & Sons. Some of these compounds have the distinct advantage over the parent steroids of possessing both sodium retaining and carbohydrate activity in high degree.

Borman, Singer and Numerof, also of the Squibb Institute, examined the series for physiological activity and report that the Chloro-F-Acetate was 10-20 times more effective than DCA in stimulating growth and survival of adrenalectomized rats and in sodium retention tests it proved to be 10.8 times more potent than DCA. The parent steroid, hydrocortisone has little activity in stimulating sodium retention except in enormous doses. Perhaps the most interesting observations of these investigators was the demonstration that the Fluoro-F-Acetate and Chloro-F-Acetate are many times more potent than cortisone in their effects on certain aspects of carbohydrate metabolism, *i.e.*, deposition of liver glycogen. Thus the glucocorticoid potency of these 9α-halo derivatives of cortisone and hydrocortisone is not only greatly enhanced by the halogen but the mineralocorticoid activity is also multiplied many times.

The Chloro-F-Acetate and Fluoro-F-Acetate were recently assayed in my laboratory on adrenalectomized dogs. The minimum maintenance dose required each day to hold the arterial pressure, body weight and serum electrolyte levels normal was 55 μg./dog/day for Chloro-F, and 27 μg./dog/day for the Fluoro-F. On the other hand the daily requirement for the non-halogenated cortisone and hydrocortisone was 5000 μg. per day. The minimum maintenance dose of DCA was approximately 250 μg. per day. These figures serve to give some idea of the greatly enhanced potency of the 9α-halo compounds.

To my knowledge, but two clinical tests of these new derivatives of cortisone and hydrocortisone have been reported to date. Thorn and his co-workers compared the potency of the Chloro-F-Acetate with hydrocortisone in a variety of metabolic and clinical studies in both Addisonian and non-Addisonian patients. Qualitatively the effects of the two compounds were the same. The Chloro-F-Acetate proved to be much more potent than hydrocortisone in tests involving circulating eosinophils and on the renal excretion of sodium, potassium chloride and uric acid. Only small doses were required to maintain the Addisonian patient. Tests of the Fluoro-F-Acetate revealed that 250 μg. per day given orally was sufficient to maintain the Addisonian patient in a near optimum clinical state.

Liddle and Associates in work on adrenalectomized dogs found the Chloro-F-Acetate to test much stronger than desoxycorticosterone or hydrocortisone and state that clinical studies confirmed the potency of compound. These drugs require further study with special reference to dosage and toxicity. Insofar as animal experimentation goes, no evidence of disabling toxicity has appeared. This has been the experience of the workers at the Squibb Institute, using adrenalectomized rats and normal dogs and the Princeton group, employing adrenalectomized dogs. According to workers in the Squibb Institute when doses of 750 μg./Kg./day are administered over extended periods the dogs develop a diabetes insipidus-like syndrome and may eliminate 3-4 L. of urine per day and ingest similar quantities of water. Despite this "water diabetes" the dogs remain active, vigorous and are otherwise free from symptoms.

Cortisone has been tested clinically on about every disease to which man is subject and a surprising number of diseases respond favorably, for a time at any rate, to the cortisone therapy. The symptoms are suppressed, the patient feels much better and may consider

himself on the high road to complete recovery. Unfortunately, however, these steroid hormones in most cases, although suppressing symptoms apparently leave the basic disease process unchanged. It will be observed that the disease states are not only numerous but are also quite diverse in character. With the exception of those disabilities listed under the heading of adrenal insufficiency in man due to gland removal, none of these conditions have been shown to be due to a defect or failure of the adrenal cortex. Although it must be conceded at this time that the basic and fundamental function of cortisone and hydrocortisone in health and disease is obscure, nevertheless we can rest assured that in the near future this problem will be solved.

Some Disease States Relieved by Cortisone and Hydrocortisone

1. Rheumatoid arthritis
2. Rheumatic fever
3. Gout
4. Bronchial asthma
5. Hay fever
6. Lupus erythematosus
7. Drug hypersensitivities
8. Periarteritis nodosa
9. Contact dermatitis
10. Ocular inflammatory disease
11. Allergic purpura of children
12. Acquired hemolytic anemia
13. Idiopathic thrombocytopenic anemia
14. Adrenal insufficiency in man
    a. Addison's disease
    b. Waterhouse-Friederichsen Syndrome
    c. Ablation adrenal glands for
        1) Removal adrenal tumor
        2) Relief severe hypertension
        3) Temporary relief prostatic and breast cancer

It would be ungracious and incorrect on this special occasion to fail to pay tribute to our colleagues in pharmacy for their contributions to steroid and other types of endocrine therapy. As with most drugs, a gap must be bridged between chemists and biologists working in the field of endocrinology and the utilization of their discoveries for

therapeutic purposes. The problem of delivering to the clinician therapeutically effective drugs, practicable in their use and stable enough to continue to exert their expected effects over long periods is primarily the responsibility of the research pharmacist. The role of these individuals in the development of new and more effective modes of administration of drugs has been very great. In this connection, witness the use of implantation pellets of steroid hormones in the long-term management of hypogonadal disabilities and Addisonian patients; parenteral suspensions of microcrystalline cortisone and hydrocortisone in treatment of rheumatoid arthritis; buccal or lingual tablets which is probably the most common dosage form for androgens. We also owe to the skill of the research pharmacist the development of stable solutions of epinephrine, elegant creams for the topical application of steroids and simple and practical methods for prolonging the action of adrenocorticotropic hormones. Both patient and physician have benefited enormously from these contributions.

To maintain a steady flow of steroid and other endocrine products to the millions requiring such therapy is a mass production task of great complexity. Here too pharmacists play a highly significant role in organizing and surveying the carefully controlled operations. The manufacturing pharmacist is a production man and the medical profession and patient are dependent upon him for large quantities of safe, potent, uniform drugs.

The distribution and handling of potent steroid and other endocrine substances requires the professional background of the trained pharmacist. Although the purely manipulatory contributions of the retail and hospital pharmacist in this field seems slight, their significant educational role in store or institution can not be overlooked. It is particularly in this complex field where dosage and regimen are of the utmost importance to the welfare of the patient that the scientific background of the pharmacist comes into play.

In closing it might be well to recall that the history of both chemistry and medicine indicate that much of the sustenance of these two branches of scientific endeavor come from pharmacy. The primary role of the pharmacist has always been the formulation, testing and distribution of medicines. As the practice of medicine, and more especially therapeutics, becomes more scientific and less empirical, the pharmacist can keep pace with the times and retain an honored place in the scientific and professional world by continued efforts in research, education and controlled distribution of useful drugs.

SELECTED ABSTRACTS

Stable Digitalis Tincture. Srivastava, G. P., Saksena, V. N., and Iyer, S. S. *The Indian Pharm. 10:66* (1954). Many factors have been proposed as those involved in the loss of potency of digitalis tincture, such as, hydrolysis, enzymatic reactions, reactions caused by microorganisms, change in hydrogen ion concentration, and varying climatic conditions.

Several tinctures were prepared by the authors in order to attempt to evaluate certain of these factors in relation to stability of the tincture. The alcohol content of either the B. P. or the U. S. P. tincture should be sufficiently high to inhibit the growth of microorganisms and enzymes. To further test this factor, sodium benzoate and sodium salicylate were added but, the loss of potency was not reduced. The adjustment of the pH to 5.4 or 5.9 had no effect on the stability. Defatting of the crude drug also had no stabilizing effect on the tincture prepared from it. Extremes of temperature of storage did not adversely affect the stability.

The authors found, however, that a mixture of absolute alcohol and glycerin in the ratio 7:3 used as the menstruum produced a tincture which was stable. The initial potency was found to be slightly lower, probably due to lower extracting power of the solvent for the digitalis glycosides, but there was no significant loss of potency upon storage. The authors concluded that the loss in potency in the official tinctures is probably due to hydrolysis of the glycosides.

Piromen Therapy in Dermatology. Lincoln, Charles S., Jr. *Med. Times 83:*178 (1955). A purified bacterial polysaccharide obtained from *Pseudomonas aeruginosa* (Piromen) was used in the treatment of various dermatological conditions. In most cases calcium gluconate was given concurrently with the indication that a synergistic relationship exists between the two substances. Piromen was given in an initial dose of 0.5 microgram intravenously or of 1 to 2 micrograms subcutaneously or intramuscularly. The dosage was increased with each subsequent visit until a clinical response or a reaction was noticed.

A total of 179 patients were treated. A variety of dermatologic conditions responded to therapy. A total of 140 cases showed good to excellent response. The response to this therapy was good in nonspecific, nummular, and allergic eczema, allergic rhinitis, dermatitis herpetiformis and several other conditions in which only a few cases were treated. The response was not good in psoriasis.

In several instances Piromen was substituted for ACTH or cortisone with a continuation of established benefit. In other cases Piromen was given instead of ACTH or cortisone. The response was not so dramatic in these cases but neither were hormonal side reactions encountered. Piromen is similar to ACTH and cortisone in that it does not provide a cure. Remissions were frequent when therapy was discontinued but, beneficial results were again obtained when therapy was re-instituted. It could be maintained with booster injections at intervals determined for the individual patient.

The incidence of side reactions such as headache, chills and fever occurred in 55 patients. However, none of the patients who improved requested discontinuation of therapy even though side reactions occurred. Reactions occurred primarily when the drug was given intravenously.

Infant Diarrhea Treated With Neomycin Sulfate. Ponce de Leon, E. *Antibiotic Med. 1*:20 (1955). A series of 25 infants up to 24 months of age were treated with neomycin sulfate for diarrhea caused by species of Shigella or Salmonella. Treatment consisted of the oral administration of 50 mg. per Kg. per day of the antibiotic in divided dosage every four hours. Treatment was continued for 5 days and was begun no later than the 4th day of illness. No other treatment had been given previously. Supportive therapy of fluid diet, oral or intravenous fluids and gradual establishment of normal feeding was given according to the age and needs of the patient.

The majority of the infants were cured clinically and bacteriologically. One infant with *Salmonella typhosa* developed the typical typhoid picture. Three patients with shigellosis also did not respond to therapy, as judged by no improvement after 4 days of treatment.

The general physical condition of the infants played an important part in the rapidity of clinical response to treatment. In no case was there evidence of toxic reactions to the drug and relapses did not occur.

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